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13. ABSTRACT   The total plasma bilirubin concentration (Br) increased by 23 to 334% in 12 individuals (10 healthy volunteers and 2 patients with Gilbert's syndrome) who fasted for 1 to 3 days. Eighty-six per cent of the rise in Br was due to an increase in plasma unconjugated bilirubin. The percentage increase in Br did not correlate with the magnitude of the base line concentration. Studies with bilirubin-^{3}H in 5 subjects showed that the hepatic clearance of bilirubin from the plasma (C_{Br}) was reduced by 28 to 54% during fasting, accounting for the increase in Br. The ratio of the plasma bilirubin turnover during fasting to that in base line state was 0.99 ± 0.16 (mean $\pm$ SD) for these five individuals, indicating that increased plasma bilirubin turnover did not contribute to the rise in Br. In 5 additional subjects the mean change in carbon monoxide production with fasting was only +24%, whereas Br increased by 188%. Further studies were done to determine why C_{Br} decreases with fasting. The plasma disappearance rate of indocyanine green in 5 subjects after a 48-hr fast was unchanged from base line. Nine homozygous Gunn rats had a $41 \pm 10\%$ (mean $\pm$ SE) increase in Br over control units with 48 hr of fasting. Bilirubin-^{3}H clearance studies in 2 homozygous Gunn rats demonstrated 43 and 44% decreases in the whole body clearance of bilirubin during fasting, with return to control rates on refeeding. Multicompartmental analysis of the human bilirubin clearance data showed that the ratio of the hepatic bilirubin pool to plasma bilirubin pool decreased during fasting. These results are most consistent with the hypothesis that C_{Br} decreases with fasting because of reduced hepatic ability to extract bilirubin from the plasma.

14. KEY WORDS	LINK A		LINK B		LINK C	
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Fasting						
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STUDIES ON THE MECHANISM OF FASTING HYPERBILIRUBINEMIA

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The total plasma bilirubin concentration (Br) increased by 23 to 334%
in 12 individuals (10 healthy volunteers and 2 patients with Gilbert's
syndrome) who fasted for 1 to 3 days. Eighty-six per cent of the rise in
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zygous Gunn rats had a 41 ± 10% (mean $\pm$ SE) increase in Br over con-
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homozygous Gunn rats demonstrated 43 and 44% decreases in the
whole body clearance of bilirubin during fasting, with return to control
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clearance data showed that the ratio of the hepatic bilirubin pool to
plasma bilirubin pool decreased during fasting. These results are most
consistent with the hypothesis that C_{Br} decreases with fasting because
of reduced hepatic ability to extract bilirubin from the plasma.

The effect of caloric intake on the serum
bilirubin concentration was noted as early
as 1906 by Gilbert and Herscher.[1] They
observed that the serum bilirubin concen-
tration was higher when individuals were
fasting. More recently, Barrett[2] and Fel-
sher et al.[3] have shown that caloric restric-
tion for 24 to 48 hr causes a significant in-

crease in the serum bilirubin concentration of both normal indi duals and patients with a variety of types of hepatic dysfunction.

This investigation was designed to determine the mechanism responsible for the hyperbilirubinemia of fasting. The results indicate that hepatic clearance of bilirubin from the plasma decreases during fasting, causing the increase in the plasma bilirubin concentration. Additional studies were done to determine why hepatic bilirubin clearance is altered by fasting.

Methods

Plasma bilirubin concentrations in human subjects were measured by the method of Weber and Schalm.[4] Plasma bilirubin concentrations in homozygous Gunn rats were measured by the method of Malloy and Evelyn[5] on 0.05 to 0.15 ml of blood collected from the tail vein in heparinized micro-blood collecting tubes (Arthur H. Thomas Co., Philadelphia, Pa.).

The plasma disappearance rate of bilirubin-^{3}H in human subjects was determined following intravenous administration of 15 to 30 μc of bilirubin-^{3}H in 0.5 mg of bilirubin,[6] using the methods of Barrett et al.[7] and Berk et al.[8] The following indices of bilirubin metabolism were calculated from parameters of the plasma disappearance curve of bilirubin-^{3}H and the plasma concentration of unconjugated bilirubin (Br)[7,8]: (1) C_{Br}, the volume of plasma cleared of unconjugated bilirubin each minute by the liver; (2) BRT, the plasma bilirubin turnover in milligrams per day; and (3) the percentage retention of labeled bilirubin in the plasma at 4 hr.

$$C_{Br} \text{ (ml per min)} = \frac{\text{initial distribution volume of bilirubin-}^3\text{H} \times 1}{\text{area under bilirubin-}^3\text{H plasma disappearance curve}} \quad (1)$$

$$
\begin{aligned}
\text{BRT (mg per day)} = &\ C_{Br} \text{ (ml per min)} \\
&\times \frac{Br}{100} \text{(mg per ml)} \\
&\times 1400 \text{ (min per day)} \quad (2)
\end{aligned}
$$

The bilirubin-^{3}H clearance data were also analyzed by multicompartmental analysis in terms of a three-pool model previously described by Berk et al.[8] In this model the plasma pool of unconjugated bilirubin is considered to exchange with a hepatic bilirubin pool and extravascular extrahepatic bilirubin pool. The pool sizes and fractional transfer rates between pools were determined.

Labeled bilirubin clearance studies in homozygous Gunn rats were done with bilirubin-^{3}H dissolved in male Sprague-Dawley rat plasma. The solution (0.5 to 1.0 ml), containing 0.1 to 0.2 mg of bilirubin, was injected into a tail vein. Plasma samples for radioactivity were obtained from blood collected at the end of the tail in heparinized micro-blood collecting tubes.

The rate of carbon monoxide production in human subjects was measured in a closed rebreathing system. The methods of Collison et al.[9] and Rodkey et al.[10] were used.

The plasma disappearance rate of indocyanine green (ICG) in human subjects was determined by the method of Cherrick et al.[11] following intravenous administration (0.5 mg per kg of body weight).

The water content of rat liver was determined as the difference between the wet weight and the dry weight of tissue. The protein content was measured on trichloroacetic acid precipitates of liver homogenates by the method of Lowry et al.[12] Bilirubin glucuronyl transferase activity in liver homogenates from male Sprague-Dawley rats was measured by the method of Black et al.[13]

Subjects

Twelve individuals participated in these studies. All individuals except H. G. and K. S. were healthy volunteers. H. G. had Gilbert's syndrome and an undefined congenital hemolytic anemia (red cell life span measured with tritiated diisopropylfluorophosphate was 30 days). K. S. had Gilbert's syndrome. Informed consent was obtained from each individual.

During periods of fasting both human subjects and rats underwent total caloric restriction, with only water intake allowed. During base line studies the human subjects were on an *ad libitum* diet, and rats were fed unlimited Purina rat chow.

Results

The plasma bilirubin concentration of each individual rose with caloric restriction (tables 1 to 3). The percentage increase in the bilirubin concentration did not correlate

with the magnitude of the base line value ($r = 0.19$). Eighty-six per cent (range 69 to 93%) of the increase in the total plasma bilirubin concentration was due to an increase in the unconjugated fraction. The greatest increase occurred during the first 24 to 34 hr of fasting, with a smaller increase over the subsequent 24 hr of fasting. The plasma bilirubin concentration in each individual returned to the base line value within 24 to 48 hr of refeeding.

The results of bilirubin-^{3}H clearance studies in 5 subjects in the base line state as compared with fasting are shown in table 1. The fasting bilirubin-^{3}H clearance study was started after 40 to 57 hr of total caloric restriction. Caloric restriction was continued for the duration of the study (an additional 24 to 30 hr). In four of the individuals the plasma bilirubin concentration had reached a new constant level by the time the fasting bilirubin-^{3}H clearance study was started (the ratios of the plasma bilirubin concentrations at the end of the study to those at the beginning were 0.89, 0.99, 1.00, and 1.09). This indicates that a new steady state with respect to the plasma bilirubin concentration was present during the fasting bilirubin-^{3}H clearance study. The plasma bilirubin concentration in H.

G. was constant over the first 15 hr of the fasting bilirubin-^{3}H clearance study, but then increased by 28% during the last 9 hr of the study. However, the measurement of hepatic bilirubin clearance was well determined in this subject (uncertainty in the measurement was ± 5%).

The difference between the plasma disappearance of bilirubin-^{3}H in the base line state compared with that during fasting is shown in figure 1. Indices of bilirubin metabolism obtained from parameters of the curves are given in table 1. In each individual the hepatic clearance of unconjugated bilirubin decreased during fasting, and the plasma retention of labeled bilirubin at 4 hr increased. The average uncertainty in the clearance measurement for the 5 subjects was ± 8% both during base line state and fasting. The decreases in hepatic bilirubin clearance (by 28 to 54%) accounted for the increases in the plasma unconjugated bilirubin concentration. The ratio of the plasma bilirubin turnover during fasting to that during base line was 0.99 ± 0.16 (mean $\pm$ SD) for the 5 subjects, indicating that an increase in plasma bilirubin turnover did not contribute to the increase in the plasma unconjugated bilirubin concentration. The increase in

TABLE 1. *Bilirubin-^{3}H clearance data*

Subject	Study	Plasma bilirubin concentration		Hepatic bilirubin clearance	Plasma 4 hr bilirubin-^{3}H retention	Plasma bilirubin turnover
		Conjugated	Unconjugated			
		mg/100 ml		ml/min	%	mg/day
C. G.	Base line	0.02 ± 0.01	0.39 ± 0.06	48	4	270
	Fasting	0.08 ± 0.02	0.86 ± 0.09	22	8	275
L. S.	Base line	0.08 ± 0.01	0.81 ± 0.06	28	11	330
	Fasting	0.24 ± 0.01	1.51 ± 0.06	18	17	391
B. C.	Base line	0.05 ± 0.01	0.97 ± 0.05	29	9	397
	Fasting	0.14 ± 0.03	1.17 ± 0.05	21	15	350
K. S.	Base line	0.07 ± 0.02	1.30 ± 0.03	15	21	285
	Fasting	0.13 ± 0.03	1.69 ± 0.12	9	37	223
H. G.	Base line	0.24 ± 0.01	8.79 ± 0.33	7	36	859
	Fasting	0.66 ± 0.07	15.47 ± 0.62	4	42	936

* Indices of bilirubin metabolism calculated from parameters of the bilirubin-^{3}H disappearance curve and the plasma concentration of unconjugated bilirubin are shown. The fasting studies in K. S. and H. G. were started after 40 hr of total caloric restriction. The fasting studies in the other three individuals were started after 57 hr of total caloric restriction.

† The plasma concentration listed for both conjugated bilirubin and unconjugated bilirubin is the mean $\pm$ SD of seven to eight samples obtained during the study.

plasma conjugated bilirubin during fasting, which forms a small percentage of the total increase, may also be due to decreased hepatic clearance, although this could not be studied by the present method.

The rate of carbon monoxide production was measured in 5 additional subjects during fasting (table 2). Carbon monoxide and bilirubin are both produced by catabolism of heme compounds.[14,15] An increase in bilirubin production resulting from increased heme degradation will be accompanied by an equimolar increase in carbon monoxide production,[16-18] although the converse may not be true.[17] Since the plasma unconjugated bilirubin concentration is directly proportional to the plasma bilirubin turnover,[19] carbon monoxide production must change by at least the same percentage as the plasma bilirubin concentration during fasting, if augmented heme degradation alone is to account for the increase. This was not observed. The mean increase in the plasma bilirubin con-

centration in the 5 subjects was 188%, whereas the mean change in the carbon monoxide production rate was only +24%. The coefficients of variation seen on several

TABLE 3. *Indocyanine green (ICG) plasma clearance*[a]

Subject	Study	Total plasma bilirubin concentration[b]	ICG plasma half life
		mg/100 ml	*min*
C. G.	Base line	0.40	3.0
	Fasting	1.12	2.8
M. C.	Base line	0.55	3.2
	Fasting	0.78	3.6
B. C.	Base line	0.81	3.6
	Fasting	1.37	3.6
J. B.	Base line	0.73	3.7
	Fasting	0.99	3.6
R. H.	Base line	0.53	4.0
—	Fasting	1.34	4.3
Mean	Base line	0.62 ± 0.16	3.5 ± 0.4
± SD	Fasting	1.10 ± 0.26	3.6 ± 0.5

[a] The plasma clearance of ICG during fasting is compared with control clearance. The base line study was done following an 8-hr overnight fast (midnight to 8 AM). Each subject was then fasted an additional 48 hr and the fasting study was performed. There was no difference in the plasma half-life of ICG during fasting compared with base line.

[b] The total plasma bilirubin concentration is the mean of at least two samples obtained during the study.

TABLE 2. *Carbon monoxide (CO) production*[a]

Subject	Study	Total plasma bilirubin concentration[b]	CO space	CO production
		mg/100 ml	*ml*	*ml/day*
J. V.	Base line	0.79	1024	12.2
	Base line	0.58	1048	14.6
	After 24-hr fast	2.01	1013	11.2
R. H.	Base line	0.63	1305	20.4
	After 48-hr fast	1.34	1415	23.5
P. B.[c]	Base line		12.5	12.8
	After 48-hr fast		1096	16.3
F. R.	Base line	0.34	824	9.2
	After 24-hr fast	0.85	794	10.5
	After 48-hr fast	0.90	771	16.9
J. R. B.	Base line	0.59	1093	13.1
	After 24-hr fast	2.05	1078	18.9
	After 48-hr fast	2.56	1111	19.0

[a] The carbon monoxide production rate during fasting is compared with that during *ad libitum* caloric intake. A closed rebreathing system was used to measure the CO production rate. All studies except those in P. B. were performed on consecutive days.

[b] The total plasma bilirubin concentration is the mean of at least two samples obtained during the study.

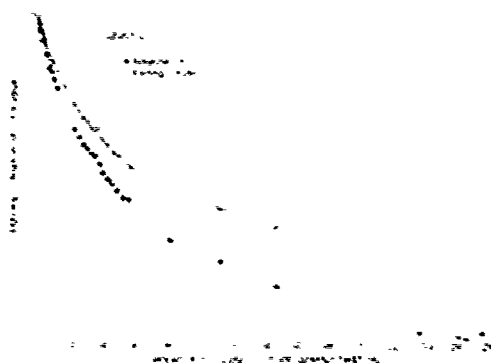[c] A base line plasma bilirubin concentration was not established.

FIG. 1. The plasma disappearance of bilirubin-³H in subject L. S. while fasting is compared with that during *ad libitum* caloric intake (base line study). Individual data *points* are shown as a fraction of the extrapolated value at zero time. The *solid lines* represent computer fits to the data. The plasma disappearance rate of bilirubin-³H decreased with fasting.

replicate determinations of the base line carbon monoxide production rate in two individuals were ±9% and ±16%. These results support the hypothesis that a reduction in hepatic bilirubin clearance is mainly responsible for the hyperbilirubinemia of fasting.

The plasma disappearance rate of ICG was determined in 5 subjects in the base line state and during fasting (table 3). Since ICG is extracted from the plasma by the liver with an efficiency of 63 to 88%, measurement of its plasma disappearance rate has been used to estimate hepatic blood flow indirectly.[11, 20, 21] In the individuals studied there was no difference in the plasma clearance of ICG in the fasting state compared with control values. These results indicate that a decrease in hepatic blood flow does not occur during fasting.

The total plasma bilirubin concentration in each of 9 homozygous Gunn rats with unconjugated hyperbilirubinemia increased during fasting. The base line concentration in these rats was 10.0 ± 3.5 mg per 100 ml (mean $\pm$ sd). During 48 hr of fasting, the mean plasma bilirubin concentration increased by 41% (range, 12 to 88%) of the
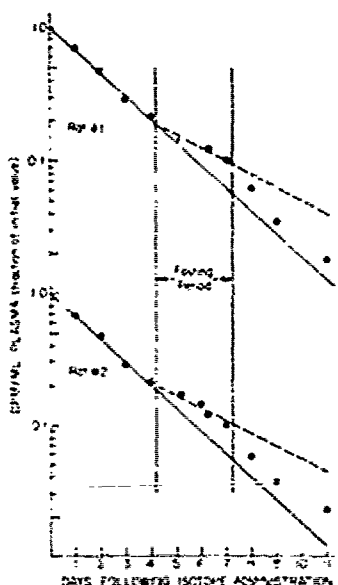

Fig. 2. The plasma clearance of bilirubin-³H in homozygous Gunn rats decreases during fasting, returning to control values on refeeding. Individual data *points* are shown as a fraction of the extrapolated value at zero time. The least squares fits to the bilirubin-³H clearance data during *od libitum* caloric intake and fasting are shown by the *solid lines* and *dashed lines* respectively.

TABLE 4. *Rat liver composition changes during fasting*[*]

Basic composition

Group	Body weight			Liver weight	Grams of H₂O per g of liver	Grams of protein per g of liver
	Base line	Fasted	Refed			
		g		*g*		
1	264 ± 20			10.2 ± 1.6	0.68 ± 0.004	0.20 ± 0.002
2	261 ± 11	226 ± 7		7.8 ± 0.7	0.69 ± 0.007	0.22 ± 0.004
3	262 ± 10	222 ± 9	249 ± 12	10.2 ± 0.8	0.68 ± 0.004	0.19 ± 0.005

Bilirubin glucuronyl transferase

Bilirubin conjugation

Group	Body weight		Liver weight	Milligrams per g of liver per hr	Milligrams per g of whole liver per hr	Milligrams per g of protein per hr
	Base line	Fasted				
		g	*g*			
4	66 ± 3		3.0 ± 0.1	2.4 ± 0.03	7.3 ± 0.3	11.7 ± 0.3
5	66 ± 1	50 ± 2	1.8 ± 0.1	2.7 ± 0.2	4.9 ± 0.6	11.5 ± 0.7
6	174 ± 6		6.5 ± 0.7	2.5 ± 0.1	15.9 ± 1.2	
7	173 ± 6	151 ± 3	4.0 ± 0.2	2.8 ± 0.2	11.2 ± 1.0	

*Seven groups of 4 rats each were used in these studies. Values listed are the mean ± sr for the entire group. Groups 1, 4, and 6 were control groups. Groups 2, 5, and 7 were studied after 48 hr of total caloric restriction. Group 3 was fasted for 48 hr, then refed for 48 hr before study.

control value. In 6 rats which were fasted an additional 24 hr there was a further increase (mean increase at 72 hr was 59% of control value). The total body weight in these rats decreased by an average of 11% during this period of fasting, whereas the mean hematocrit rose slightly from 40% to 41%.

Labeled bilirubin clearance studies in 2 homozygous Gunn rats are shown in figure 2. Rat 1 was a 240-g female with a total plasma bilirubin concentration of 15.7 mg per 100 ml. Rat 2 was a 196-g female with a bilirubin concentration of 14.6 mg per 100 ml. The fractional turnover of the total miscible bilirubin pool, determined from the terminal slope of the plasma disappearance curve, was 0.397 per day in rat 1 and 0.401 per day in rat 2 during normal caloric intake. The rate decreased to 0.226 per day in rat 1 and to 0.223 per day in rat 2 during fasting, with return to control rates on refeeding. These results in an animal which lacks bilirubin glucuronyl transferase indicate that a decrease in the rate of bilirubin conjugation is unlikely to explain the increase in the plasma unconjugated bilirubin concentration which occurs during fasting.

In male Sprague-Dawley rats a decrease in both total body weight (by 15 to 24%) and liver weight (by 24 to 40%) occurred during 48 hr of fasting (see table 4). The concentration of protein in the liver rose approximately 15% during fasting ($P < 0.01$ by Student's t-test). The water content [grams of H_2O per gram of liver (wet weight)] did not change. Identical changes in hepatic protein and water content occurred in Gunn rats during fasting. The changes in bilirubin glucuronyl transferase activity in male Sprague-Dawley rat livers were the same as for total protein, and activity per gram of protein did not change.

The bilirubin-^{3}H clearance data from human subjects were subjected to multicompartmental analysis in an attempt to define the specific step in hepatic bilirubin excretion which is altered by fasting.[8] The model used (see fig. 3) considers the plasma pool of unconjugated bilirubin (M_1) to exchange with a hepatic pool (M_2) and ex-
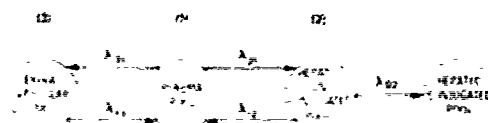

Fig. 3. Three compartment model for the metabolism of unconjugated bilirubin. Values for the λ's, which are the fractional transfer rates between compartments, and for the pool sizes are calculated from the plasma disappearance curve of bilirubin-^{3}H and the plasma concentration of unconjugated bilirubin.

TABLE 5. *Multicompartmental analysis of bilirubin-^{3}H clearance studies*[a]

Subject	Study	λ_{21}	λ_{12}	λ_{r2}	$M_2{:}M_1$	$M_3{:}M_1$
C. G.	Base line	0.032	0.015	0.015	1.86	3.50
	Fasting	0.019	0.016	0.016	0.59	2.67
L. S.	Base line	0.015	0.008	0.009	0.50	2.48
	Fasting	0.012	0.016	0.013	0.40	1.96
B. C.	Base line	0.011	0.001	0.005	1.67	1.68
	Fasting	0.012	0.012	0.014	0.45	1.74
K. S.	Base line	0.010	0.006	0.006	3.82	1.19
	Fasting	0.005	0.004	0.004	0.63	1.30
H. G.	Base line	0.006	0.009	0.006	0.42	1.00
	Fasting	0.005	0.014	0.006	0.27	0.85

[a] Results of multicompartmental analysis of bilirubin-^{3}H clearance data in human subjects are shown. λ_{21} = the fractional transfer rate of unconjugated bilirubin (FTR) from plasma pool to hepatic pool. λ_{12} = FTR from hepatic pool to plasma pool. λ_{r2} = irreversible fractional removal rate of unconjugated bilirubin from hepatic pool (= conjugation). $M_2{:}M_1$ = ratio of hepatic pool of unconjugated bilirubin (milligrams) to plasma pool (milligrams). $M_3{:}M_1$ = ratio of extravascular extrahepatic pool of unconjugated bilirubin (milligrams) to plasma pool (milligrams).

travascular extrahepatic pool (M_3) of unconjugated bilirubin. The fractional transfer rates between pools are depicted on figure 3 as λ's. In each individual a decrease in the ratio $M_2:M_1$ occurred during fasting, indicating that the ability of the liver to extract bilirubin from the plasma was reduced by fasting (table 5). This decrease in the ratio of pool sizes was associated with a decrease in the fractional transfer rate of bilirubin from plasma to liver (λ_{21}) and/or an increase in the fractional transfer rate from liver to plasma (λ_{12}). The factional rate which represents conjugation, λ_{v2}, decreased by 33%, in subject K. S. during fasting but was unchanged or increased in the other 4 subjects. The ratio $M_3:M_1$ was unchanged in 2 subjects during fasting compared with base line, and slightly decreased in the other 3 subjects.

Discussion

The hyperbilirubinemia of fasting may be due to either a decrease in hepatic clearance of bilirubin from the plasma or an increase in plasma bilirubin turnover resulting from increased bilirubin production (or a combination of both). In this investigation evidence has been presented which indicates that a reduction in hepatic bilirubin clearance during fasting is principally responsible for the increase in the plasma bilirubin concentration.

Measurements of the rate of carbon monoxide production in human individuals during fasting (table 2), while supporting the hypothesis that reduced hepatic bilirubin clearance is mainly responsible for the hyperbilirubinemia of fasting, suggest that an increased rate of heme degradation may also occur. Bakken et al.[22] have in fact shown in rats that the activity of hepatic heme oxygenase, the enzyme responsible for the conversion of heme to bilirubin and carbon monoxide, is increased by fasting and by hormones released during hypoglycemia. The production of carbon monoxide-^{14}C from heme labeled with glycine-2-^{14}C is also increased.[23] The increased rate of carbon monoxide production observed in our subjects during fasting

may thus indicate an increased rate of hepatic heme degradation. If this also reflects increased hepatic bilirubin production, the portion of hepatic bilirubin production which circulates in the plasma prior to excretion in the bile would contribute to the hyperbilirubinemia of fasting.[24] It is necessary to point out, however, that an increase in the rate of carbon monoxide production does not always mean that there is an increase in bilirubin production. Landaw et al.[17] showed in rats with experimental porphyria that hepatic heme may be degraded along nonbilirubin pathways. Likewise, Schacter et al.[25] have described a mechanism for the degradation of heme and hemoprotein which results in the formation of carbon monoxide but variable amounts of bilirubin.

The physiological alteration which causes the decrease in hepatic bilirubin clearance during fasting seems to be independent of the base line clearance rate and plasma bilirubin concentration. In 2 subjects with Gilbert's syndrome (K. S. and H. G. in table 1), base line hepatic bilirubin clearance was well below the normal range previously described.[5] Three other individuals (C. G., L. S., and B. C.) had values for clearance which were within the normal range. Subject L. S. had a plasma bilirubin concentration which was only 10% of that for H. G., and his base line hepatic bilirubin clearance was 3-fold greater. Yet both individuals had nearly identical percentage changes in both the plasma bilirubin concentration and hepatic bilirubin clearance with fasting. Multicompartmental analysis of the bilirubin clearance data demonstrated that the ratio of the hepatic bilirubin pool to plasma bilirubin pool decreased in each individual during fasting (table 5).

It is unlikely that hepatic bilirubin clearance decreases with fasting because of a decrease in hepatic blood flow. In order to explain the magnitude of the increase in the plasma bilirubin concentration, a marked reduction in hepatic blood flow would be necessary. This would cause a decrease in the plasma clearance of ICG, since the plasma disappearance rate of ICG offers an indirect measurement of he-

patic blood flow which correlates very well with direct measurements.[11, 26, 21] Such a decrease was not observed in five individuals (table 3).

A decrease in the activity of hepatic bilirubin glucuronyl transferase is also unlikely to explain the hyperbilirubinemia of fasting. The demonstration that the plasma bilirubin concentration increases with fasting in Gunn rats which lack this enzyme, direct measurement of bilirubin glucuronyl transferase activity in homogenates of male Sprague-Dawley rat liver (table 4), and multicompartmental analysis of human bilirubin clearance data (table 5) indicate that a decrease in the activity of this enzyme does not explain the increase in the plasma bilirubin concentration during fasting.

Two hepatic cytoplasmic protein fractions which bind bilirubin and other organic anions have been described.[26] However, the turnover rates of these proteins (half-times of 19 days and 42 hr)[27] are too long to account for the rapidity of rise in the plasma bilirubin concentration during fasting, even if synthesis of the proteins stopped with fasting.

Diminished excretion of bilirubin into bile during fasting could cause the increase in plasma conjugated bilirubin. However, this would not explain the increase in plasma unconjugated bilirubin, since only conjugated bilirubin is excreted into bile.

A mechanism to explain fasting hyperbilirubinemia which is consistent with all the data presented is that a material forms (or increases) during fasting which inhibits hepatic bilirubin clearance. Alternatively, a material which facilitates hepatic bilirubin clearance could be depleted. Several compounds which may influence bilirubin metabolism change rapidly with fasting. For example, the plasma glucagon level increases to a peak by 3 days of fasting, whereas the plasma insulin and glucose levels decrease over the same time period.[26] This is thought to reflect a period of accelerated hepatic gluconeogenesis. If hepatic uptake of bilirubin is energy-dependent, such changes might be important. Free fatty acids, which have been shown to

affect the binding of bilirubin to albumin, increase markedly within 24 hr of fasting and return rapidly to base line values on refeeding.[28] Although the plasma concentration of free fatty acids attained during fasting is not sufficient to cause displacement of bilirubin from albumin in vitro,[26] the free fatty acids might compete effectively with bilirubin for the hepatic cytoplasmic binding proteins, since the liver actively metabolizes free fatty acids during fasting.

Many other changes occur with fasting, and further work will be required to define the reason why hepatic bilirubin clearance decreases. Such investigation should add to the knowledge of bilirubin metabolism.

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